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PHARMACOKINETICS AND METABOLISM OF DICHLOROACETIC ACID AND TRICHLOROACETIC ACID ADMINISTERED IN DRINKING WATER IN RATS AND MICE

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Interim Report – January 1997 – January 1999

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This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

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As by-products of water chlorination, dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are ubiquitous contaminants of drinking water supplies. They are also major metabolites of several heavily used industrial solvents. DCA is a complete hepatocarcinogen and a tumor promoter in B6C3F1 mice. TCA is a tumorigen in mice and affects cardiac development in Sprague-Dawley rat fetuses. To describe the pharmacokinetics of orally ingested DCA and TCA, male Fischer 344 rats and B6C3F1 mice were given DCA in drinking water at 3 different dose levels (rat - 0.1, 0.5 and 2.0 g/l; mice - 0.08, 0.8, and 2.0 g/l) for 3 or 14 days. Blood and liver samples were collected at selected time points up to 8 hr after removal from treated drinking water. Cytosol was prepared from the remaining rat and mouse livers for investigation of metabolic competency. At the end of 14 days of treatment the blood DCA concentrations for mice and rats were 18.5 and 73.3 ug/ml, respectively, at the 2.0 g/l dose level. Liver concentrations at the same time point and dose level for mice and rats were 8.3 and 11.6 ug/ml, respectively. At the 0.08 and 0.8 g/l DCA dose level DCA was not measured in mouse blood or liver. Peak blood concentrations of TCA were 4.3, 30.6 and 93.6 ug/ml in rats drinking 0.1, 0.5 and 2.0 g/l of TCA, respectively, and 10.3, 72.9 and 79.9 ug/ml in mice drinking 0.08, 0.8 and 2.0 g/l of TCA, respectively. A group of mice and rats that received DCA treated drinking water were intravenously dosed with DCA after removal of the treated drinking water to determine inhibition of DCA metabolism. Alteration of metabolic degradation of DCA was substantial at the 0.8 and 2.0 g/L drinking water dose levels in both rats and mice.

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PREFACE

This technical report describes the pharmacokinetics and metabolism of halogenated chloroacetic acids administered in drinking water to rodents. The pharmacokinetics and metabolism of dichloroacetic acid and trichloroacetic acid administered in drinking water are detailed in this report. This research was performed at the Operational Toxicology Branch, Human Effectiveness Directorate of the Air Force Research Laboratory. The study was supported by ManTech – Geo-Centers Joint Venture Contract #F41624-96-C-9010 and funded by the US Environmental Protection Agency IAG # DW57937914-01-0.

The animal use described in these studies was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1996, as amended.

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ABSTRACT

As by-products of water chlorination,dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are ubiquitous contaminants of drinking water supplies. They are also major metabolites of several heavily used industrial solvents. DCA is a complete hepatocarcinogen and a tumor promoter in B6C3F1 mice. TCA is a tumorigen in mice and affects cardiac development in Sprague-Dawley rat fetuses. To describe the pharmacokinetics of orally ingested DCA and TCA, male Fischer 344 rats and B6C3F1 mice were given DCA in drinking water at 3 different dose levels (rat – 0.1, 0.5 and 2.0 g/l; mice - 0.08, 0.8 and 2.0 g/l) for 3 or 14 days. Blood and liver samples were collected at selected time points up to 8 hr after removal from treated drinking water. Cytosol was prepared from the remaining rat and mouse livers for investigation of metabolic competency. At the end of 14 days of treatment the blood DCA concentrations for mice and rats were 18.5 and 73.3 ug/ml, respectively, at the 2.0 g/l dose level. Liver concentrations at the same time point and dose level for mice and rats were 8.3 and 11.6 ug/ml, respectively. At the 0.08 and 0.8 g/l DCA dose level DCA was not measured in mouse blood or liver. Peak blood concentrations of TCA were 4.3, 30.6 and 93.6 ug/ml in rats drinking 0.1, 0.5 and 2.0 g/l of TCA, respectively, and 10.3, 72.9 and 79.9 ug/ml in mice drinking 0.08, 0.8 and 2.0 g/l of TCA, respectively. A group of mice and rats that received DCA treated drinking water were intravenously dosed with DCA after removal of the treated drinking water to determine inhibition of DCA metabolism. Alteration of metabolic degradation of DCA was substantial at the 0.8 and 2.0 g/L drinking water dose levels in both rats and mice. These results may play an important role in interpreting DCA and TCA and drinking water dose-response studies that have been conducted with rodents.

Keywords: dichloroacetic acid, trichloroacetic acid, drinking water, pharmacokinetics, metabolism, inhibition

INTRODUCTION

Dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are byproducts of water chlorination and are ubiquitous contaminants in finished drinking water (Quimby et al., 1980, Uden and Miller, 1983). DCA has been use to treat diabetes (Stacpoole and Green, 1992) and hypercholesterolemia (Moore et al., 1979). However, DCA has been shown to cause central nervous system toxicity and to induce hepatic tumors in rodents (Stacpoole et al., 1979; Bull et al., 1990, DeAngelo et al., 1991; Richmond et al., 1995). DCA acts as a complete hepatocarcinogen in mice (Herren-Freund et al., 1987). TCA is a hepatic carcinogen in mice (Herren-Freund et al, 1987; Bull et al, 1990; Daniel et al, 1992; Pereira, 1996), but not in rats, although histopathological changes were seen in rat liver (DeAngelo et al, 1991). TCA is poorly metabolized and is long lived in animals and humans. Larson and Bull (1992) reported that 50 to 65% of administered TCA is excreted in urine by 24 hr in rats and 48 to 55% of administered TCA is excreted in mice. In rats about 16 to 20% of the administered TCA is metabolized by 24 hr and about 12 % of TCA is metabolized in mice by 24 hr.

Both DCA and TCA are metabolites of several chlorinated solvents (Larson and Bull, 1992) and are toxicologically significant metabolites of trichloroethylene (TCE), a widely used industrial solvent and problematic groundwater contaminant. Laboratory bioassays have demonstrated TCE's ability to induce hepatocarcinomas in rodents, resulting in TCE's classification as a B2 carcinogen (USEPA, 1985; NTP, 1990). It is believed, however, that the metabolic products of TCE, such as DCA and TCA, are responsible for its toxicity and carcinogenicity (Bruckner, 1989; Larson and Bull, 1992; Moore and Harrington-Brock, 2000). The USEPA has given priority status to the re-evaluation of TCE, and much of the focus of the research has been on its metabolites. The USEPA has classified DCA as a B2 probable human carcinogen and TCA as a "C" rodent carcinogen.

Several drinking water studies have been performed with DCA. In some of these studies DCA caused a non-linear, threshold-like dose response in male and female B6C3F1 mice (Bull et al., 1990; DeAngelo et al., 1991). Interestingly, it has also been reported that DCA inhibits its own metabolism in Fischer 344 rats and B6C3F1 mice (Gonzalez-Leon et al., 1997, 1999). Metabolism of DCA produces oxalic acid, glyoxalic

acid and glycolic acid. Chronic toxicity and carcinogenicity studies with TCA have been conducted as drinking water studies, as well (Herren-Freund et al., 1987; Bull et al., 1990; Daniel et al., 1993; Pereira, 1996). Little data is available regarding the kinetics of DCA or TCA administered in drinking water. The data presented here will be used to support the development and validation of a PBPK model that will provide a more science-based approach to determine the risks to humans from orally ingested DCA and TCA.

MATERIALS AND METHODS

Drinking Water – Experimental:

Male B6C3F1 mice and Fischer 344 rats (Charles Rivers Laboratories, Raleigh, NC) were maintained in polycarbonate cages with hardwood chip bedding. All were housed 1 per cage. Food and water were available *ad libitum*. Treated mice (n=5/time point) were provided with drinking water containing 0.08, 0.8 or 2.0 g/L of DCA or TCA neutralized to pH 7 with NaOH. Treated rats (n=6) were provided with drinking water containing 0.1, 0.5 or 2.0 g/L DCA or TCA neutralized to pH 7 with NaOH. Control animals for each dose group were given on drinking water containing sodium chloride at the same concentration as that dose group. Drinking water was contained in 60 mL glass tubes capped with rubber stoppers. The drinking tubes were designed to prevent dripping. Water bottles were weighed daily to determine volume of consumption and dose rate. Animals were divided into two groups: 3 day and 14 day. Three day animals were weighed on days 1 and 3; 14 day animal were weighed on days 1, 7 and 14. After 3 or 14 days animals were taken off treated drinking water and given RO water. Mice were sacrificed by CO_2 asphyxiation at various time points up to 8 hr post-treatment. Blood samples were collected via the inferior vena cava, and whole livers were removed. Rats were bled at various times points up to 8 hr via the lateral tail vein using a 25 G needle fitted with a heparinized capillary tube. At the last time point the rats were sacrificed by CO_2 inhalation, and blood and liver were collected as with the mice. For all animals a 200 mg portion of the liver was removed for quantification of DCA or TCA while the remainder was frozen in liquid nitrogen for cytosolic preparation.

Quantification of DCA and TCA:

200 μL of blood were mixed with 200 μL of 20% lead acetate to quench any metabolism (Ketcha et al, 1996) and stored at –80°C. 200 mg liver samples were quenched with 400 μL of 20% lead acetate, homogenized and stored at –80°C pending analysis. DCA and TCA was quantified in the blood and liver samples by gas chromatography (Abbas and Fisher, 1997). The limit of detection for DCA and TCA was 0.05 ug/mL.

Quantification of Oxalate, Glyoxalate and Glycolate:

Blood samples from animals treated with DCA in drinking water were analyzed for oxalate, glyoxalate and glycolate by the HPLC method of Narayanan et al. (1999).

I.V. Oxalate Dosing:

Male B6C3F1 mice (n=5/time point) were intravenously injected through the lateral tail vein with 10 or 50 mg/kg oxalic acid neutralized with NaOH. The animals were sacrificed by CO_2 inhalation at 0.25, 1, 2, 3, 4 and 8 hr post dose. Blood was collected from the inferior vena cava and analyzed for oxalate concentration as described above.

In Vitro Inhibition of DCA Metabolism:

Unperfused livers from rats and mice treated with DCA in drinking water were thawed, and cytosol was prepared following the method of Guengerich et al (1991). The cytosol was analyzed for DCA concentration to determine the residual amount of DCA. Cytosol from the 0.08 and 0.8 g/l dose groups had no detectable levels of DCA. Cytosol from the 2.0 g/l dose group had a residual level of 1 μg/ml DCA. Protein concentration was determined using the Pierce BCA protein assay kit. Liver cytosol (100 μl) diluted to 4 mg/ml protein was incubated with 100 μl of DCA solution, 100 μl of NADPH Regenerating System and enough 0.1 M phosphate buffer to bring the total volume to 1 ml. Incubations were carried out in 4 ml amber vials at 37°C for 10 min. The DCA concentrations ranged from 1 to 40 μg/ml. To quench the reaction at 10 min 100 μl of

the incubate were added to 100 µl of 20% lead acetate. The quenched samples were derivatized for DCA analysis by gas chromatography as described above. A Lineweaver-Burke plot was generated to determine the metabolic constants Km and Vmax.

In Vivo Inhibition of DCA Metabolism:

Male B6C3F1 mice were placed on DCA treated drinking water (0.08, 0.8 and 2.0 g/l) for 14 days. All conditions were the same as described above. At the end of 14 days the mice were removed from the treated drinking water and were given RO water, *ad libitum*. At time zero (after removal from treated drinking water) the mice (n = 5/time point) were dosed intravenously through the lateral tail vein with 50 mg/kg DCA that had been neutralized with NaOH. The animals were sacrificed by CO_2 inhalation at 0.25, 0.5, 0.75, 1, 2 and 3 hr post i.v. injection. Blood and liver were taken for DCA analysis as described above.

Statistical Analysis:

Statistical analysis was performed using a 2-tailed t-test with pooled error. The minimum significance level was $p < 0.05$.

RESULTS

A. Dichloroacetic acid

Body weights and water consumption for control and treated mice and rats are shown in Table 1. Changes in body weight for the treated mice were not different than control mice at all dose levels. All mice gained weight over time. The volume of water consumed by control and mice receiving 0.08 g/l DCA was the same. Water consumption for the 0.8 g/l group was significantly less than the control group on day 3 (using day 14 controls). Three out of 5 rats on 2.0 g/L DCA drinking water lost weight over the 3 day period, and 2 out of 5 rats lost weight over the 14 day period. Overall, rats on 2.0 g/l DCA for 14 days gained significantly less weight than control rats or rats on 0.5 or 0.1 g/l DCA drinking water. All rats consumed significantly less water than control rats, regardless of dose group. The daily dose rate (mg/kg/day) for mice and rats

is also shown in Table 1. The daily dose rate is based on average rodent body weight and average daily water consumption.

DCA was not detected in blood or liver of mice dosed with 0.08 or 0.8 g/l DCA in drinking water after 3 or 14 days of treatment. After 14 days of 2.0 g/l DCA in water the DCA concentration in mouse blood was 18.5 ± 6.9 μg/ml and in liver at 8.3 ± 3.5 μg/ml immediately after removal of treated water (Figure 1). The concentration of DCA in both blood and liver rapidly decreased and was non-detectable at 8 hr.

Table 1. Body weights and water consumption for control and DCA treated rodents.

	3 day			14 day		
	A*	B	C	A	B	C
DCA treated mice						
BW (g) – initial	26.3 ± 1.1	27.7 ± 1.7	---	26.6 ± 1.2	27.9 ± 1.7	26.7 ± 0.9
BW (g) – final	26.7 ± 1.0	27.9 ± 1.7	---	28.8 ± 1.8	29.7 ± 1.8	28.4 ± 1.0
Weight change	0.4 ± 0.4	0.2 ± 0.4	---	2.2 ± 1.0	1.8 ± 1.1	1.7 ± 0.7
Daily water Consumption – ml	4.1 ± 0.4	3.3 ± 0.5	---	4.3 ± 0.6	4.0 ± 0.8	4.1 ± 0.3
Daily dose rate mg/kg/day	12.58	103.23	---	11.67	110.44	297
DCA treated rats						
BW – initial	243.1 ± 14.8	252.7 ± 4.8	244.9 ± 8.7	241.8 ± 7.3	248.6 ± 6.8	242.2 ± 18.2
BW – final	250 ± 7	257.6 ± 6.4	249.6 ± 15.9	260.7 ± 7.2	276.8 ± 10.9	244.1 ± 20.5
Weight change	6.9 ± 12.7	4.9 ± 2.5	4.7 ± 20	18.9 ± 2.2	28.2 ± 7.6	1.8 ± 8.7
Daily water Consumption – ml	21.3 ± 6.3	19.7 ± 7.9	16.9 ± 3.5	19.0 ± 3.5	19.9 ± 4.6	15.0 ± 4.2
Daily dose rate mg/kg/day	9.26	49.24	144.79	6.81	34.41	108.8
Control Mouse						
BW (g) – initial	---	---	---	26.6 ± 0.1	27.4 ± 2.0	25.3 ± 1.4
BW (g) – final	---	---	---	30.4 ± 1.8	28.7 ± 1.6	27.5 ± 2.1
Weight change	---	---	---	3.8 ± 1.6	1.3 ± 1.5	2.2 ± 1.4
Daily water Consumption – ml	[illegible]	[illegible]	4.8 ± 0.4	4.3 ± 1.0	4.2 ± 0.6	4.0 ± 0.5
Control Rat						
BW – initial	-----	-----	-----	282.8 ± 64.8	296.9 ± 35.4	290.6 ± 39.6
BW – final	-----	-----	-----	299.9 ± 56.9	315.4 ± 23.9	312.7 ± 34.9
Weight change	-----	-----	-----	17.2 ± 8.0	18.5 ± 14.5	22.1 ± 5.1
Daily water Consumption – ml	-----	-----	-----	21.2 ± 3.5	22.6 ± 3.1	23.9 ± 4.6

* For mouse: A = 0.08 g/l, B = 0.8 g/l, C = 2.0 g/l; For rat: A = 0.1 g/l, B = 0.5 g/l, C = 2.0 g/l

All values are represented as mean ± s.d. except daily dose rate.

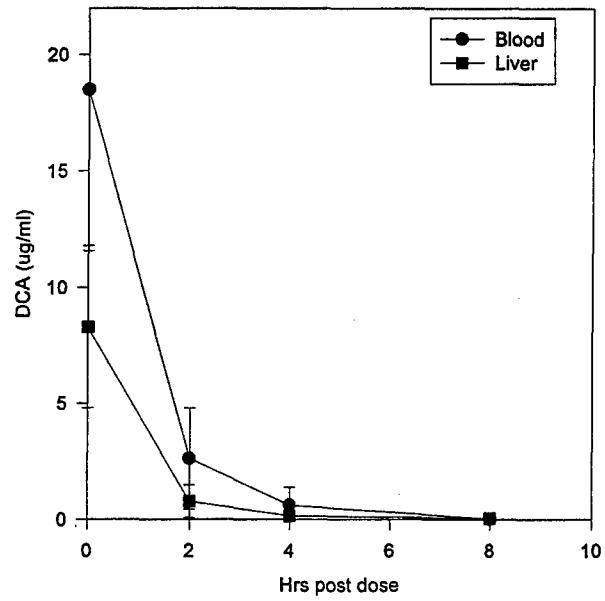

Figure 1. Clearance of DCA from blood and liver of male B6C3F1 mice after 14 days of 2.0 g/l DCA in drinking water. Data are represented as mean ± s.d. N = 5 for each time point.

Blood and liver from rats exposed to 0.1 g/l DCA in drinking water for 3 or 14 days did not have any detectable levels of DCA. After 3 days blood DCA were 29.4 ± 5.3 μg/ml and 139.2 ± 29.8 μg/ml for the 0.5 and 2.0 g/l dose levels, respectively, immediately after removal of treated water (Figure 2). The blood levels of DCA cleared slowly and detectable amounts of DCA were still present after 6 and 8 hr. DCA was still present in liver from the 0.5 g/l dose at 6 hr and in liver from the 2.0 g/l dose level at 8 hr, the last time points studied. After 14 days of 0.5 g/l DCA treated water blood levels of DCA at time 0 were 21.4 ± 3.7 μg/ml and cleared slowly to 9.0 ± 1.6 μg/ml at 6 hr (Figure 3). Blood from rats exposed to 2.0 g/l DCA for 14 days had a DCA concentration of 73.3 ± 32.5 μg/ml at 0 hr. The blood levels of DCA cleared more rapidly than blood levels on day 3, yet were still detectable (26.2 ± 15.5 μg/ml) at 8 hr. Liver DCA concentration was 2.8 ± 1.1 μg/ml at 6 hr for the 0.5 g/l level and 11.6 ± 8.6 μg/ml at 8 hr for the 2.0 g/l dosage.

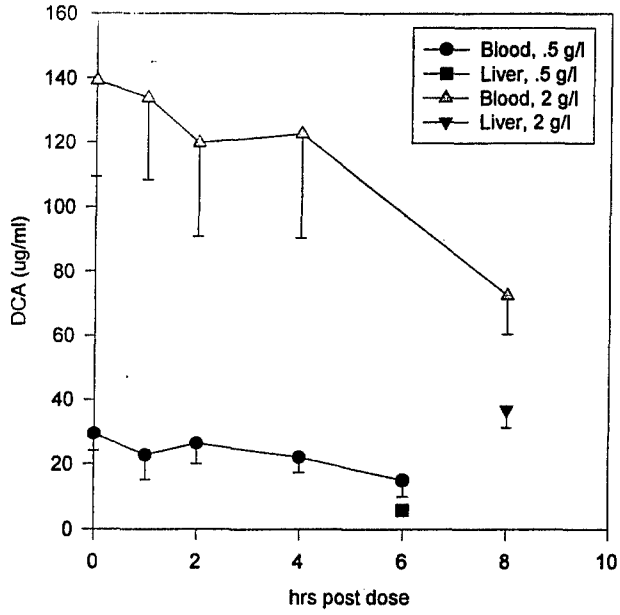

Figure 2. Clearance of DCA from blood of male F344 rats after 3 days of DCA in drinking water. Liver DCA concentration at sacrifice 6 or 8 hrs after removal from treated drinking water. Data are mean ± s.d. N = 5 for each time point.

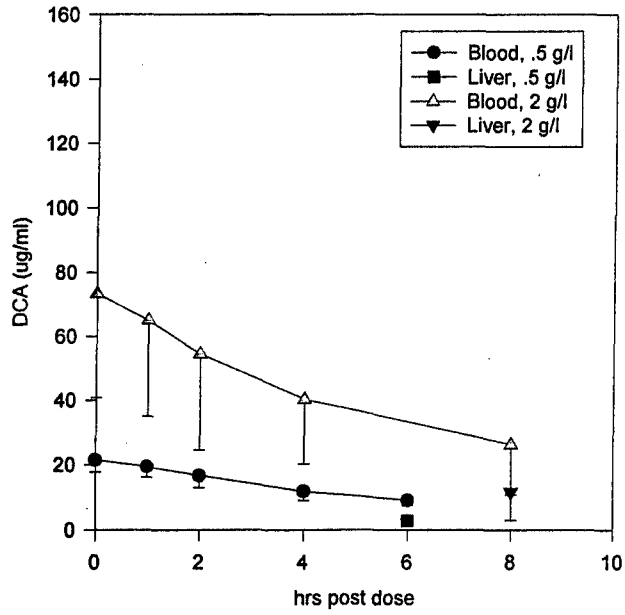

Figure 3. Clearance of DCA from blood of male F344 rats after 14 days of DCA in drinking water. Liver DCA concentration at sacrifice 6 or 8 hrs after removal from treated drinking water. Data are mean ± s.d. N = 5 for each time point.

Blood taken from mice on DCA treated drinking water was analyzed by HPLC for the metabolites oxalate, glyoxalate and glycolate. Only oxalate was present in detectable, reliable amounts. Figure 4 shows the clearance of oxalate in blood over 8 hr after removal from DCA treated drinking water. Blood oxalate concentration was the highest in the 0.08 g/l DCA, 14 day treatment group at 35.9 ± 6.4 μg. Oxalate cleared to nearly non-detectable levels by 8 hr post DCA dose. To understand the kinetics of oxalate, mice were intravenously dosed with oxalic acid neutralized with NaOH. At the 10 mg/kg dose blood levels of oxalate was 38.1 ± 3.1 μg/ml 15 min post injection and cleared rapidly (Figure 5). Oxalate was near the limit of detection by 4 hr post dose. At the 50 mg/kg dose blood levels were 366.1 ± 47.5 μg/ml at 15 min post-injection. Clearance from blood was very slow with 258.3 ± 15.4 μg/ml still remaining after 8 hr. The concentration of oxalate in control mouse blood (n = 11) was 0.63 ± 1.5 μg/ml.

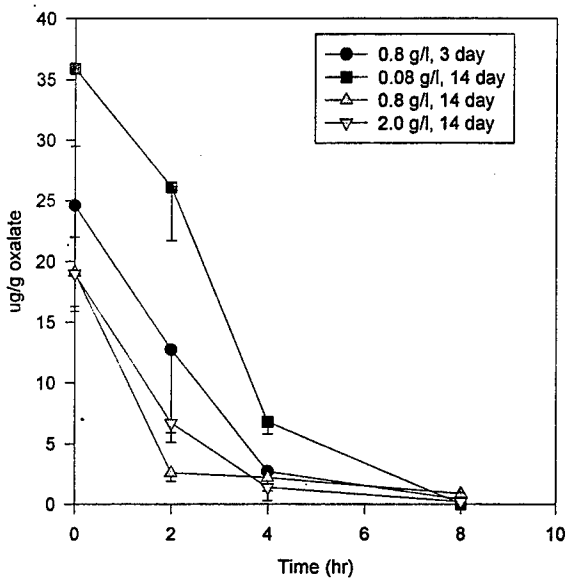

Figure 4. Blood oxalate levels in male B6C3F1 mice after treatment with DCA in drinking water for 3 or 14 days. Data are mean ± s.d. N = 5 for each time point.

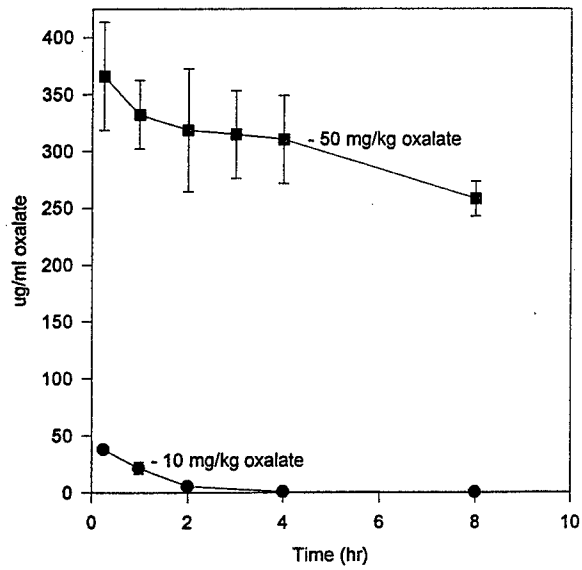

Figure 5. Blood oxalate levels in male B6C3F1 mice not treated with DCA after i.v. dose of 10 or 50 mg/kg oxalate Data are mean ± s.d. N = 5 for each time point.

Previous studies have shown that DCA inhibits its own metabolism in rodents and humans (Gonzalez-Leon et al., 1997, 1999; Curry et al., 1991). To investigate this effect cytosol was prepared from the livers of mice exposed to DCA in drinking water for 14 days and rats exposed to DCA for 3 and 14 days. The residual amount of DCA in the prepared cytosol was determined and is shown in Table 2. The cytosol was incubated with a range of DCA concentrations. The Km and Vmax values for control mice and rats did not differ substantially from the 0.08 and 0.1 g/l dose levels (Table 3). However, there was more than an order of magnitude of difference in Km and Vmax between the control, 0.5, 0.8 and 2.0 g/l dose levels.

Table 2. Residual DCA in rat and mouse liver cytosol.

	Dose level	# days	ug/ml DCA
Rat	0.1	3	ND
		14	ND
	0.5	3	1.7
		14	1.1
	2	3	10
		14	3.2
Mouse	0.08	14	ND
	0.8	14	ND
	2	14	1

Table 3. In vitro inhibition of DCA metabolism.

	3 day			14 day			
	A*	B*	C*	A*	B*	C*	Control
Mouse							
Km, nmol	---	---	---	138.7	25.7	6.6	324.7
Vmax, nmol/min/mg	---	---	---	3.0	0.39	0.43	7.8
Rat							
Km, nmol	749.7	5.47	0.58	218.2	9.4	5.0	206
Vmax, nmol/min/mg	5.84	0.41	1.69	1.02	0.32	0.78	3.79

* For mouse A = 0.08 g/l, B = 0.8 g/l, C = 2.0 g/l; For rat A = 0.1 g/l, B = 0.5 g/l, C = 2.0 g/l

To investigate further the inhibition of DCA metabolism mice were intravenously injected with 50 mg/kg DCA after being on treated drinking water for 14 days. Blood levels of DCA in mice treated with 2.0 g/L DCA in drinking water were 8.9 ± 4.4 ug/ml at 15 min. The blood levels of DCA rapidly decreased to the limit of detection by 1 hr. The blood levels of DCA in mice that were intravenously dosed with 50 mg/kg DCA were 18.5 ± 6.9 μg/ml at 0 hr and cleared more slowly than blood levels after treatment in drinking water. However, in mice that were iv dosed with 50 mg/kg DCA after treatment with 2.0 g/l DCA in drinking water for 14 days, blood levels of DCA were substantially higher at 76.5 ± 17.0 at 15 min.

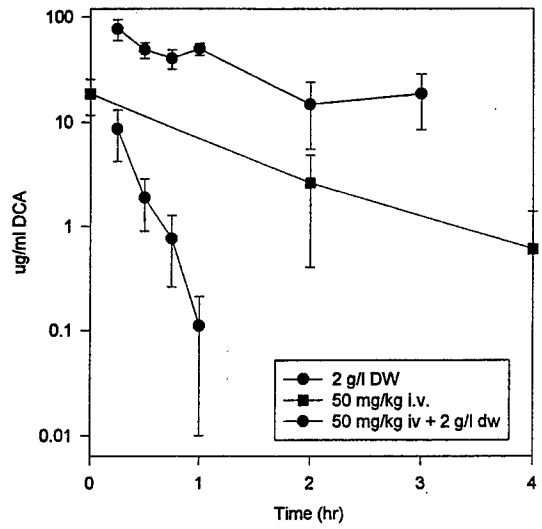

Figure 6. Blood levels of DCA in male B6C3F1 mice after i.v. dose of 50 mg/kg DCA. Mice were treated with DCA in drinking water for 14 days prior to the challenge dose. I.v. injection was performed immediately after removal of mice from treated drinking water. Data are mean ± s.d. N = 5 for each time point.

B. Trichloroacetic acid

Body weights and water consumption for control and treated mice are shown in Table 4. After 3 days on TCA treated drinking water the body weights of the mice on 0.8 and 2.0 g/l TCA were significantly less than the control group (14 day). Water consumption for mice on 0.8 and 2.0 g/l TCA in water for 3 or 14 days were significantly less than the control group ($p \leq .05$). Only the rats on 2.0 g/l TCA for 14 days had a significant reduction in water consumption. The daily dose rate (mg/kg/day) for rats and mice are shown in Table 4. The daily dose rate is based on average rodent body weight and average daily water consumption.

Figures 7a, b show the clearance of TCA from blood of mice for 24 hr after removal from treated drinking water after 3 or 14 days. Blood concentrations of TCA immediately at the end of exposure were 10.3, 72.9 and 79.9 ug/ml in mice drinking 0.08, 0.8 or 2.0 g/l of TCA for 14 days, respectively. Virtually the same concentrations in mouse blood were reached after only 3 days on treated drinking water. Liver

concentrations of TCA at 0 hr were 6.2, 48.2 and 61.6 ug/ml in mice drinking 0.08, 0.8 or 2.0 g/l of TCA, respectively, after 14 days on treated water (Figure 8a, b).

Figure 9 depicts the clearance of TCA from blood of rats after removal from treated drinking water after 3 days. Liver time course data is not available. After 3 days on treated drinking water, rat blood levels of TCA at the end of exposure were 3.5, 30.4 and 96.4 ug/ml for the 0.1, 0.5 and 2.0 g/l dose groups, respectively. The TCA liver concentrations at sacrifice on day 3 were 0.8 ±.32, 7.3 ± 0.25 and 13.0 ± 2.6 μg/ml for the 0.1, 0.5 and 2.0 g/l TCA dose groups, respectively.

After 14 days on treated drinking water rat blood TCA concentrations were nearly the same as after 3 days. Blood TCA concentrations at 0 hr were 4.3, 30.6 and 93.6 ug/ml in rats drinking 0.1, 0.5 and 2.0 g/l TCA (Figure 10). Liver time course data is not available. On day 14 the TCA liver concentrations at sacrifice were 1.4 ± 0.1, 6.2 ± 1.2 and 11.6 ± 3.8 μg/ml for the 0.1, 0.5 and 2.0 g/l TCA dose groups, respectively.

Table 4. Body weights and water consumption for control and TCA treated rodents.

	3 day			14 day		
	A*	B	C	A	B	C
TCA treated mice						
BW (g) – initial	26.6 ± 1.2	28.1 ± 1.3	27.1 ± 1.2	26.7 ± 1.1	28.4 ± 1	27.4 ± 1.2
BW (g) – final	27.7 ± 1.2	28.4 ± 1.6	27.3 ± 1.3	29.7 ± 1.8	28.7 ± 1.6	29.1 ± 1.6
Weight change	1.1 ± 0.5	0.4 ± 0.9	0.2 ± 1.5	3.0 ± 1.5	0.2 ± 1.4	1.7 ± 0.8
Daily water Consumption – ml	3.9 ± .8	3.5 ± 1.2	3.4 ± 1.2	4.2 ± .7	3.8 ± .8	3.6 ± .5
Daily dose rate mg/kg/day	13	111.5	272.1	11.6	110	268
TCA treated rats						
BW – initial	225.9 ± 8.9	230.8 ± 8.4	232.1 ± 6.5	232.8 ± 7.4	231.9 ± 6.8	224.7 ± 9.5
BW – final	242.4 ± 16.4	237.3 ± 7.1	235.2 ± 6.9	263 ± 9.8	263.8 ± 10.3	251.2 ± 11
Weight change	16.5 ± 16.4	6.5 ± 2.1	3.2 ± 3.5	30.1 ± 2.6	31.9 ± 6.4	26.5 ± 2.8
Daily water Consumption – ml	22.5 ± 3.5	22.5 ± 2.5	21.9 ± 9.8	21.3 ± 6.1	21.0 ± 6.1	17.1 ± 3.4
Daily dose rate mg/kg/day	9.8	49.1	149.7	6.8	36.4	108.3
Control Mouse						
BW (g) – initial	---	---	---	26.6 ± 0.1	27.4 ± 2.0	25.3 ± 1.4
BW (g) – final	---	---	---	30.4 ± 1.8	28.7 ± 1.6	27.5 ± 2.1
Weight change	---	---	---	3.8 ± 1.6	1.3 ± 1.5	2.2 ± 1.4
Daily water Consumption – ml			4.8 ± 0.4	4.3 ± 1.0	4.2 ± 0.6	4.0 ± 0.5
Control Rat						
BW – initial	-----	-----	-----	282.8 ± 64.8	296.9 ± 35.4	290.6 ± 39.6
BW – final	-----	-----	-----	299.9 ± 56.9	315.4 ± 23.9	312.7 ± 34.9
Weight change	-----	-----	-----	17.2 ± 8.0	18.5 ± 14.5	22.1 ± 5.1
Daily water Consumption – ml	-----	-----	-----	21.2 ± 3.5	22.6 ± 3.1	23.9 ± 4.6

* For mouse: A = 0.08 g/l, B = 0.8 g/l, C = 2.0 g/l; For rat: A = 0.1 g/l, B = 0.5 g/l, C = 2.0 g/l

All values are represented as mean ± s.d. except daily dose rate.

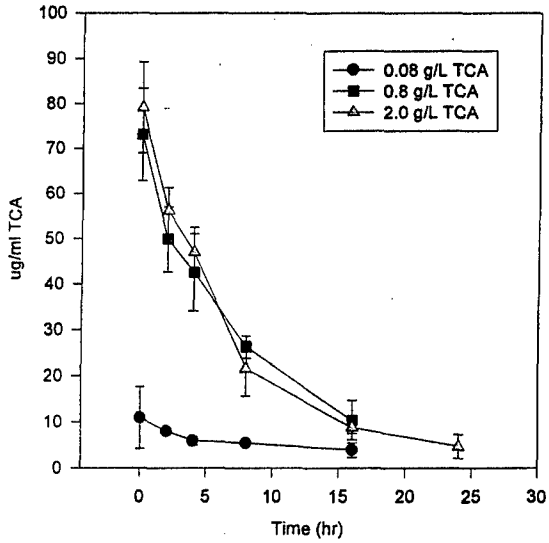

Figure 7a. Clearance of TCA from blood male B6C3F1 mice after 3 days on treated drinking water. Data are mean ± s.d.

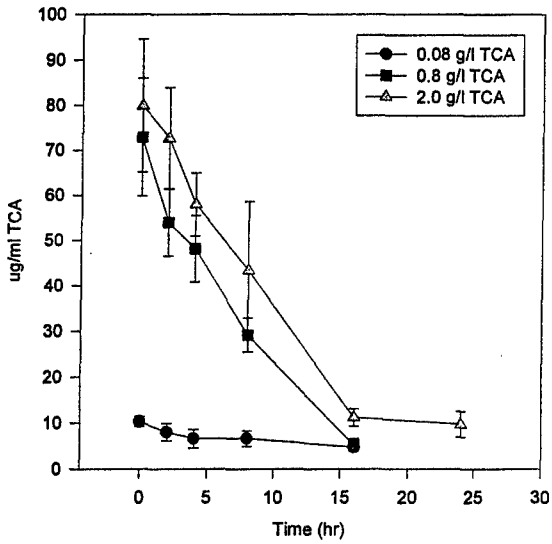

Figure 7b. Clearance of TCA from blood of male B6C3F1 mice after 14 days on treated drinking water. Data are mean ± s.d.

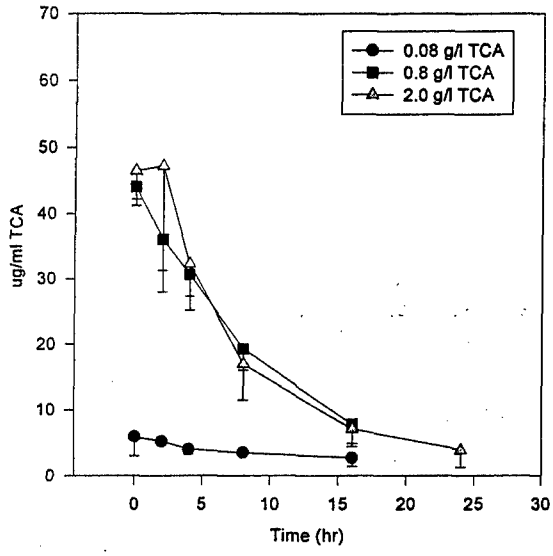

Figure 8a. Clearance of TCA from liver of male B6C3F1 mice after 3 days on treated drinking water. Data are mean ± s.d.

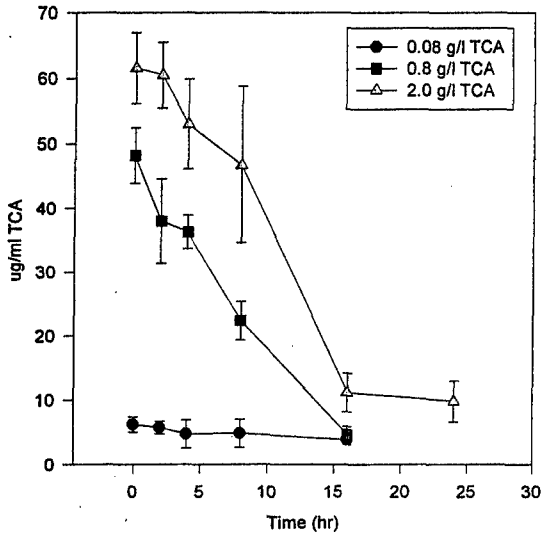

Figure 8b. Clearance of TCA from liver of male B6C3F1mice after 14 days on treated drinking water. Data are mean ± s.d.

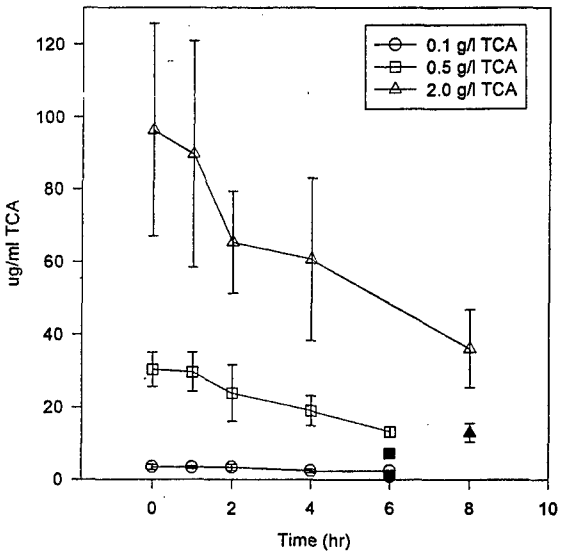

Figure 9. Clearance of TCA from blood of male F344 rats after 3 days on treated drinking water. Closed symbols are liver TCA concentrations at sacrifice 6 or 8 hrs after removal from treated drinking water. Data are mean ± s.d.

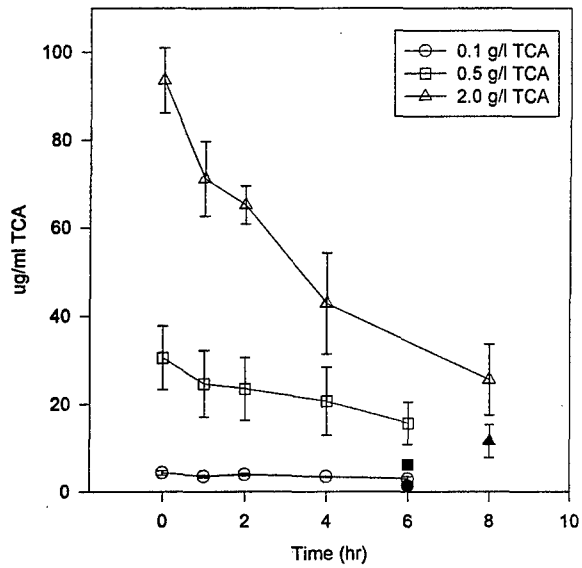

Figure 10. Clearance of TCA from blood of male F344 rats after 14 days on treated drinking water. Closed symbols are liver concentrations at sacrifice 6 or 8 hrs after removal from treated drinking water. Data are mean ± s.d.

DISCUSSION

There is sufficient evidence to suspect DCA of being a carcinogen. There is a lack, however, of sufficient kinetic data to develop and validate a physiologically based pharmacokinetic model capable of providing more information to a science-based approach to determining the risk to humans of orally ingested DCA.

Liver is likely the target organ for DCA. Understanding the distribution of DCA in blood and liver after oral ingestion is important for model development. DCA was not detected in blood or liver of mice dosed with 0.08 or 0.8 g/l DCA in drinking water. Measurable concentrations of DCA were found in both blood and liver of mice treated with 2.0 g/l. DCA is cleared rapidly from blood and liver; the half-life appears to be less than an hour. With such a short half-life the DCA probably did not accumulate in blood or liver at the 0.08 or 0.8 g/l dose levels, and any DCA present was most likely below the limit of detection.

The distribution of DCA in blood and liver of rats exposed to DCA was slightly different than mice. DCA was not detected in tissues after a dose of 0.1 g/l for 3 or 14 days. Although substantial amounts of DCA were present in blood and liver, it did not clear as rapidly from rat tissue as it did from mouse tissue. In fact, it took at least 4 hr post exposure for the concentration in blood to clear to half the level of the initial concentration for both 3 and 14 day treatments. This is interesting since previous work has suggested that DCA causes more liver damage in mice than rats.

DCA is known to metabolize to oxalate, glyoxalate and glycolate. In these studies only oxalate was reliably detectable. When mice were i.v. dosed with 10 mg/kg oxalate the blood concentration of oxalate reached approximately the same levels as blood oxalate derived from DCA metabolism at the 0.08 g/l dose level. In both cases oxalate cleared rapidly. When mice were i.v. dosed with 50 mg/kg oxalate blood levels cleared very slowly

Gonzalez-Leon et al (1997, 1999) reported that prior doses of DCA altered the disposition and pharmacokinetics of subsequent doses of DCA in rats and mice. They observed this effect both *in vivo* and *in vitro*. The same inhibition of DCA metabolism was seen in these experiments, both *in vivo* and *in vitro*. The control Km and Vmax values for mouse liver cytosol reported here are comparable to those reported by Gonzalez-Leon (1999). At the 2.0 g/l DCA treatment level, Km and Vmax values varied by at least 1 order of magnitude, however. There was a clear dose-response relationship between the treatment dose level and subsequent DCA metabolism in the *in vivo* studies.

The evidence supporting TCA's potential for carcinogenicity is limited and conflicting. Several drinking water studies have been done to investigate the carcinogenicity of TCA. In one study, uninitiated mice that were treated with 5 g/l TCA developed hepatocellular carcinomas in 32% of animals (Herren-Freund et al., 1987). Bull et al. (1990) reported a dose-related increase in hepatocellular carcinomas in mice treated with TCA in drinking water. The doses ranged from 1 to 2 g/l over the course of 12 months. F344 rats did not develop hepatic tumors when treated with TCA administered in drinking water (DeAngelo et al., 1997).

In the drinking water studies reported here the disposition of TCA in blood and liver were investigated and described. TCA cleared rapidly from blood and liver of mice exposed to 0.08, 0.8 and 2.0 g/l TCA. By 24 hr the TCA was at or near limits of

detection. Interestingly, in mice the apparent peak blood concentration of TCA was nearly the same when TCA was administered for 3 or 14 days. However, the apparent peak liver concentration was slightly higher, though not significantly, in the 0.08 and 0.8 g/l dose groups. At the 2.0 g/l TCA dose level, the apparent peak liver concentration in mice is 32 % higher after 14 days than after 3 days, suggesting that TCA is accumulating in the liver.

The rat blood levels of TCA show a dose response relationship after 3 and 14 days of treatment. TCA cleared rapidly from blood of rats administered 0.1, 0.5 or 2.0 g/l TCA. Liver data is only available at the sacrifice time point. However, the concentration of TCA in rat liver at 6 and 8 hr is essentially the same after 3 and 14 days of ingestion of treated drinking water, indicating no accumulation in the liver.

Understanding the kinetics of DCA and TCA is important to understanding the risks that they pose to human health. The distribution of DCA and TCA to the target tissues, primarily the liver, affects its ultimate toxicity. Being able to describe the pharmacokinetics of orally ingested DCA and TCA in a physiologically based model can help interpret DCA and TCA and drinking water dose-response studies that have been conducted with rodents.

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